THE

AMERICAN REVIEW OF TUBERCULOSIS
ABSTRACTS OF TUBERCULOSIS

VOLUME XX JULY, 1929 ABST. No. 1

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Cellular Reactions to Derivatives of Tubercle Bacillus.—In another article the authors have shown that clasmatocytes and monocytes react differently toward tubercle bacilli. While both phagocyte bacilli, the clasmatocyte on the one hand fragments the bacteria readily and rapidly, as it does all debris with which it has to deal, while the monocyte retains the specific bacillus intact so long that the relationship may be considered one of symbiosis. In the present paper the response of these phagocytic cells to the protein and phosphatide (phospholipin) fractions from the tubercle bacillus is studied. The amount of material available limited the administration of the protein fractions to 7 rabbits, and the phosphatide fractions to 5 rabbits, with a control group consisting of 6 rabbits. Both proteins (304 and 903) were given intravenously. Protein 903, found to be more toxic than the other, was administered in smaller dosage. The results with the protein showed that both fractions were toxic, both produced anaemia with neutrophilia of greater or less degree, an increase in clasmatocytes in the lung, multiple haemorrhagic foci scattered throughout, from diaphragm to pelvis, focal fatty necrosis of the liver cells, and some damage to the cells of the renal tubules. The phosphatide fractions (A-3 and A-4) in watery emulsion were administered intraperitoneally. The dose represented the proportion of this material in 80 mgm. of dry bacilli. The phosphatide fractions showed only one response, namely the local production in the peritoneal cavity of the typical lesion of tuberculosis. There was a massive local increase in monocytes, epithelioid cells, and Langhans giant cells; there was some infiltration of this tubercular tissue with lymphocytes, and there was some necrosis. If the observations that in the disease itself clasmatocytes fragment the living bacilli, and that the clasmatocytes are in turn disintegrated and the resulting debris rephagocytized, actually mean that large numbers of bacilli are killed and broken down by the cells of the animal, then the actual progress of a tuberculous infection involves the effects of both living and dead bacilli on the body. The control series were divided into three groups: one received lecithin; another received the same dosage of lecithin plus a known added weight of dead human tubercle bacilli; the third received dead bacilli alone. All were injected intraperitoneally. The protein series was not controlled, as adequate bacterial material was not available. These control intraperitoneal injections caused a local cellular stimulation,

predominantly clasmatocytic in all the animals but one. The dead bacilli alone in repeated doses excited only a very limited development of the cellular changes characteristic of tuberculosis; lecithin was found quite incapable of inducing other than a nonspecific phagocytic response; and the combination of lecithin and dead bacilli reacts essentially independently with only a minimal response of epithelioid cells. *Summary: 1:* The clasmatocyte, the cell with the power of fragmenting tubercle bacilli, the cell making the lesion of the so called interstitial pneumonia, has been shown to be the overwhelming response to the special protein fractions. *2:* The epithelioid and the giant cell of the Langhans type, making typical tuberculous tissue, have been the massive and specific response of the peritoneal cavity to intraperitoneal injection of the phosphatide fractions.—*The Biological Reactions in Rabbits to the Protein and Phosphatide Fractions from the Chemical Analysis of Human Tubercle Bacilli, Florence R. Sabin and C. A. Doan, J. Exper. Med., October 1, 1927, xlvi, 645.—(J. S. W.)*

Origin of Pulmonary Dust Cell.—The article begins with a fairly full review of the present status of the doctrine of the dust cell (*Herzfehlerzell*), which includes the early work of the author, Gross's résumé, and the work of the Westheuses, Lewis, Sabin, Maximow and others. The remainder of the article is devoted to the original experiments of the author and a critical discussion of the subject. In order to attack the problem from several angles at once, the following experiments were performed: Rabbits were given intravenous and intratracheal injections of various dyes and milk; the cells that responded in the alveoli were examined *in vivo* in supravital films; and the lungs were sectioned, after freezing or after embedding in paraffin and examined from the morphological standpoint. A specific method for identifying monocytes, histiocytes and dust cells on the one hand, and distinguishing them from epithelium or from pleural mesothelium on the other, was fortunately found. This is the so called specific silver-tannate technique, in which paraffin sections are first mordanted in a solution of tannic acid, and ammonium-bromide in formalin. Sections are then impregnated in a weak silver-ammonium-oxide solution, and washed and "toned" in a special preparation of Merck's "brown acid" gold-chloride. They are then dehydrated and mounted in the usual manner. In sections stained in this way the dust cells become reddish brown, with brownish black to sepia granules, the latter being usually grouped in rosettes and balls as in typical monocytes. In blood smears, impregnated with silver-tannate, the monocytes and polymorphonuclears show the specific dark brown cytoplasm and sepia granules seen in the sections. The lymphocytes do not take the granular stain, their cytoplasm being almost colorless, but their nuclei stand out distinctly. In the first experiment carmine-red was injected intravenously into a rabbit, and 25 cc. of its heart's blood were withdrawn after a wait of fifteen minutes. This blood was then citrated and injected intravenously into each of three rabbits in equal parts. These next received intratracheal injections of from 3 to 4 cc. of pasteurized milk containing 0.5 per cent of saturated alcoholic Sudan-III to cause some pulmonary irritation. The rabbits were killed at twenty-four, forty-eight and ninety hours, respectively, by introducing air into the circulation. Scrapings from the lungs were given supravital staining, and the lungs were then fixed and sectioned. By introducing "marked monocyte" from another animal, it was hoped that the blood origin of the intraälveolar phagocytes might be proved or disproved; if these contained carmine it is obvious that they would most probably represent marked cells from the donor animal, unless polymorphonuclear leucocytes should have emigrated and been engulfed by the dust cells, in which case uncertainty would result. However, the carmine injected could not be found in the blood cells of the animals that received the transfused blood. In the second rabbit experiment less milk was given intratracheally to cause less reaction in the bronchi. The observations tallied in the main with the preceding experiment. No carmine marked cells were found in any of the sections. The pulmonary dust cells all showed the characteristic silver-tannate staining, which puts them in the class with the monocytes. Paraffin sections from human lungs showing chronic passive congestion exhibited, even more strikingly than the rabbit, the specific character of the silver-tannate impregnation for dust cells. From evidence adduced from the literature and the experiments here described, most likely origin of the pulmonary dust cell appears to be from the blood-stream, and, more specifically, the monocytes of the latter. That various reticuloendothelial elements, such as the supporting cells of the lymphoid tissue of the lung, the adventitial cells of its vessels or even lymphocytes themselves, share to a degree in the production of the dust cells cannot be denied; that they play the chief part in the production, however, seems improbable. That the vascular endothelium gives rise to dust cells under

ordinary conditions (a belief previously advocated by the author) seems entirely unlikely in the face of evidence accumulated during the past decade. In view of this fact it will be necessary to readjust our theories of inflammation so that they may more nearly conform with those of Metschnikoff and the Lewises on the one hand and of Maximow on the other. The dust cells are probably large forms of monocytes or blood histiocytes. While the tissue histiocytes may play some part in their production, it is more likely that the ranks are recruited from the circulating monocytes of the blood-stream. The origin of these is still disputed. The alveolar epithelium of the lung does not produce dust cells in so far as it can be ascertained; it possesses totally different affinities for silver salts, and can, by means of silver-tannate impregnation, be readily recognized and differentiated from the alveolar macrophages (dust cells), which appear to be of mesenchymal, rather than of endodermal origin. Furthermore, the reaction of these groups to supravital stains are equally divergent. The assumption that there is no nucleated "respiratory epithelium" does not appear to be warranted, for sections supravitally stained with neutral-red and counter-impregnated with silver-tannate show pictures in every way similar to those drawn by anatomical investigators. As the refractive index of alveolar epithelium is very close to that of glass, it is imperative that some procedure be used that will render it artificially visible, or that the light in the condenser be arranged so as to utilize the very slightly higher refractive index of the epithelium, otherwise it will escape notice.—*Studies on Endothelial Reactions. X. On the Origin of the Pulmonary "Dust Cell," N. C. Foot, Am. J. Path., September, 1927, iii, 413.*—*(J. S. W.)*

Origin of Alveolar Phagocyte.—The problem of the origin of the alveolar phagocyte is approached from an entirely new angle. The use of supravital staining with neutral-red, which has accomplished so much in the reclassification of blood and exudate cells, has hitherto been limited to smears and scrapings. A new method has been devised to preserve supravital staining with neutral-red in paraffin sections, thus preserving the topographical relationship of the stained cells. Animals are killed by air embolism, and the dye is introduced either into the air-spaces through the trachea or into the vascular system. This technique has been employed to study the histogenesis of the alveolar phagocyte. Intravenous staining affects chiefly the cells in the framework of the lung; intratracheally it not only reacts upon the cells, but is sufficiently irritating to provoke a very rapid migration of alveolar phagocytes. From the character of the cytoplasmic reactions to neutral-red it has seemed possible to identify the alveolar phagocytes with the "septal cells," which project above the surface of the epithelium lining the air-spaces. These septal cells are in turn classified as a form of clasmatocyte or connective-tissue phagocyte. From the study of a series of guinea pigs and rabbits (mostly the former) by these improved methods, it is concluded that the septal cells rapidly desquamate because of the presence of the irritating dye in the air-spaces. The epithelial cells are not affected but remain in position. Within the alveoli the desquamated septal cells undergo morphological and physiological modifications resulting in the formation of typical alveolar phagocytes. New septal cells, replacing those which have migrated from the alveolar walls, are regenerated from connective-tissue monocytes, which in turn arise from dormant reticulum cells stimulated by the presence of the irritating dye. In support of these views the following observations are presented: Within five minutes after intratracheal staining the alveoli contain large numbers of phagocytes indicating a readily accessible source of supply. Such a source might conceivably be found in the following locations: (1) cells already in the aveoli, (2) cells in the circulating blood, and (3) cells in the permanent structure of the lung. The first source, preëxisting alveolar cells, can be eliminated, first, because phagocytes appear too rapidly for cell division to have taken place, and, second, because the experiments were repeated on guinea pigs only one day old, whose lungs, not being previously irritated by inhaled dust, do not contain free phagocytes. The second source, cells in the blood-stream, can also be eliminated because the number of available cells remaining in the pulmonary vessels after the circulation has failed is too small to supply all the free phagocytes which are observed. There remains, then, only some cell in the permanent structure of the lung as a source of alveolar phagocytes. The fixed-tissue elements, which have been suggested as possible origins, are alveolar epithelium, capillary endothelium and connective-tissue phagocytes or clasmatocytes. The reactions to neutral-red of the first two types in no way resemble those of the alveolar phagocyte. Epithelial cells, studied in thick section, exhibit only a few fine granules widely scattered throughout the cytoplasm; endothelial cells do not take up the dye at all, unless they are so damaged that the nucleus is stained. Furthermore,

there is no evidence of any depletion of these cells after intraälveolar irritation. Their number remains the same before and after intratracheal injection of neutral-red. The clasmatocytes of the connective tissue increase in number with the duration of irritation. They are apparently derived from extra vascular monocytes, which are in turn produced by stimulation of dormant reticulum cells (Sabin). Clasmatocytes are found to some extent in the heavy areolar tissues of the trunks, and are very abundant in the alveolar septa. In the latter position they often project into the lumen of the air-space between the epithelial cells. They are characterized by great numbers of neutral-red granules, varying in size and color, scattered throughout their cytoplasm. After the dye has been injected into the trachea of the recently killed animal, the number of clasmatocytes in the alveolar septa is notably decreased. This is not so obvious in the living animal where regeneration is constantly taking place. Because of the similarity between the staining of free phagocytes and those in the connective tissues, and because of the depletion of the latter by the intratracheal injection of irritants, the authors favor this cell as the usual source of alveolar phagocytes. However, it is not impossible that some of the immature connective-tissue monocytes may migrate directly into the air-spaces. This form, which is usually smaller than that in the blood, has been found in the alveoli, although it is rare. Its arrangement of neutral red granules in a rosette even more closely resembles that seen in the early free phagocytes than in the cells of the septa. *Conclusions: 1:* The alveolar phagocyte is produced by the desquamation of septal cells. *2:* The septal cell belongs to the general group of connective-tissue phagocytes of clasmatocytes. *3:* These clasmatocytes appear to arise from previously inactive reticulum cells which pass through a monocyte-like stage in the process of maturation. *4:* Alveolar epithelium and local vascular endothelium play no part in the origin of the alveolar phagocyte. *5:* The arrangement of the neutral-red-stained cytoplasmic elements is not fixed and cannot therefore be used as a criterion to differentiate clasmatocytes from monocytes.—*The Origin of the Alveolar Phagocyte Studied in Paraffin Sections of Tissue Stained Supravitally with Neutral Red, L. U. Gardner and D. T. Smith, Am. J. Path., September, 1927, iii, 445* and *579.*—(*J. S. W.*)

Primary Pulmonary Focus.—As a result of an examination of 18 cases of the primary complex in children during the ages of 0 to 12 years, only once was there a single focus found in the apex, twice there were 2, twice there were 3, and once 9 foci, while once a primary cavity existed. Histologically the centre showed a caseous pneumonia. Encapsulating connective tissue consisted not only of specific epithelioid-cell tissue but also of nonspecific, vascular, rich granulation tissue. The perifocal changes consisted not only of haemorrhagic manifestations but also of strangulated bronchi and vessels. The hilum catarrh of Ranke was found relatively rarely. Chronic bronchitides were rare. As a whole, the Ranke teaching of the primary complex was verified.—*Untersuchungen über den primäre tuberkulösen Lungenherd, E. Schulze, Beitr. z. Klin. d. Tuberk., 1928, lxviii, 216.*—(*H. J. C.*)

Onset of Pulmonary Tuberculosis in Adults.—Recent publications make it necessary to sound a warning against too schematic concepts in the development of pulmonary tuberculosis. A case is reported in which a middle-field lesion on the right side was the first clinical manifestation. Since the infiltrative process excavated, artificial pneumothorax treatment was given for two years. The patient made a complete recovery. Later on she developed a chronic apical tuberculosis on the left side, which healed. After a delivery, complicated by eclampsia, she developed an infraclavicular infiltration on the left side. This lesion showed evidence of excavation. This case demonstrates that an apparently early infraclavicular infiltration may in reality be a late manifestation, that an apparently early cavity may be a late cavity, and that an apical iesion may develop after a subapical lesion has healed.—*Zum Beginn der Lungentuberkulose des Erwachsenen, K. Henius, Klin. Wchnschr., October 7, 1928, vii, 1690.*—(*M. P.*)

Origin of Chronic Pulmonary Tuberculosis.—The original hypothesis of Ranke that tuberculosis, from primary infection to adult phthisis, runs its course in three successive stages (primary allergy, secondary allergy, immunity) is no longer tenable. All three stages may occur at any age and all three may be observed during adult phthisis at the site of each new lesion. In adults the secondary allergic exudative reaction is always less prominent than the proliferative reaction when immunity is increasing, and more prominent when this phenomenon is waning. Apical lesions in adults are the result of dissemination of bacilli by the blood-stream from an older focus, and are most obvious at the apex because haematogenous areas in other parts

of the lung tend to regress while those at the apex grow, and because of repeated focalization of blood-borne bacilli at the apex (?). They may heal but they may become reactivated. Necrosis and cavity-formation are of great importance because closed lesions are nearly all benign. It has been shown (Braeuning) that only 7 per cent of closed apical tuberculosis becomes open after three years, while of 248 open cases only 13 per cent had a previous history of apical tuberculosis. Other observers have been convinced that most apical lesions do not go on to open tuberculosis, although it must not be presumed that such a course is not possible. Phthisis is very often the end-result of an activation of an infraclavicular infection, which may go on to perifocal inflammation and cavity-formation. Such areas of disease may also heal, however. This type of disease may arise from aerogenous superinfection, although it may also be haematogenous. This infraclavicular, progressive tuberculosis is the commonest source of adult phthisis. In the early stages it may give rise to few signs and many symptoms. However, very early the sputum is bacillary and the X-ray indicates the area and is important. —*Neue Anschauungen ueber die Entstehung der chronischen Lungenphthisie, A. Bacmeister, Deutsche med. Wchnschr., December 16, 1927, liii, 2151.*—(*H. S. W.*)

Spread of Tuberculosis in Body.—Far larger numbers of ingested bovine tubercle bacilli are required to produce disease than inhaled human tubercle bacilli. The explanation probably lies in the cul-de-sac structure and relative stasis in the bronchopulmonary unit, and in the activities of the alveolar phagocytes. Most patients with lupus develop the lesion through bacilli carried there by the circulation. Penetration of an intact mucous membrane by the tubercle bacillus is probably rare, but on penetration of an abraded membrane there follow phagocytosis and eventually infection of a lymph node. Primary tuberculosis of the nasal mucous membrane is almost unknown, although the bacilli are frequently found in the nasal passages. Primary tuberculosis may occur in the tonsils, bacilli being carried within by phagocytes. The lymph nodes, or second line of defense, probably cannot overcome even a moderate number of tubercle bacilli, and if the bacilli escape, to cause disease elsewhere in the body, there are always enough in the nodes to cause disease there. Autopsy investigation in children has shown that the respiratory portal of entry predominates over the alimentary in the ratio of 53 to 25. Meningitis is part of a generalized tuberculosis and more commonly associated with thoracic than with abdominal disease. It is a fairly frequent terminal complication of male genital tuberculosis, especially after surgical intervention. Bone and joint tuberculosis may originate from either thoracic or abdominal disease, the blood-stream being the only probable route of metastasis here. The bacilli are probably not distributed directly from one lymph-node chain to another in the case of distant metastasis, but by means of the general circulation. Occasionally bovine tuberculosis attacks the mesenteric lymph nodes while the tracheobronchial nodes remain normal; but it is doubtful whether choroid, tonsillar, lingual, pharyngeal or laryngeal tuberculosis ever occurs without involvement of the tracheobronchial nodes. Tuberculosis of the larynx, pharynx and trachea is probably most frequently caused by bacilli carried by the blood-stream, rather than by surface infection from heavily infected sputum. Data from autopsy records may be abnormally high as the result of technical breakdown of the defensive mechanism. If surface infection were the most important factor, lesions of the superficially exposed organs should be the commonest complications. While tuberculosis of the larynx occurs in about 25 per cent of cases of pulmonary tuberculosis, Calmette states that tubercles can be found in the liver in nearly all persons dead of tuberculosis, and in the spleen about as often. It has been proved that bacillaemia without acute general miliary tuberculosis occurs in a considerable number of cases of pulmonary tuberculosis, and it has been shown that tuberculous enteritis can be produced experimentally by actual inoculation much more readily than by simple infection. It is therefore reasonable to suppose that tuberculous enteritis and laryngitis do have a haematogenous origin, at least in many cases.—*The Distribution and Extension of Tuberculosis, G. B. Webb, J. Am. M. Ass., September 15, 1928, xci, 769.*—(*G. L. L.*)

Pathological Anatomy of Healed Pulmonary Tuberculosis.—This study was undertaken in order to establish the frequency and anatomical character of tuberculous alterations in patients who had not died of tuberculosis, including only such lesions which may at some time have had some clinical significance. Primary lesions were not considered. After eliminating patients with marked and active tuberculous processes, 238 cases remained for study. Fifty-seven cases were found (24 per cent) which showed isolated parenchymal lesions whose size never exceeded that of a pigeon's egg. Most of them were found in the apical

fields. With the exception of 12 cases multiple foci were found. The anatomical character of the various foci was as follows: scar-formation 41, caseous 7, fibrocaseous 10, cavitation 8. These figures show that apical tuberculosis is not as frequent as usually represented from an anatomical point of view and not as harmless as claimed in recent literature. Manifestations of what Hollò has called juvenile tuberculosis were found in 57 cases. In this group were included all tuberculous alterations which were evidently due to haematogenous generalization without establishment of a tertiary phthisis, such as multiple healed pulmonary lesions, pleural scars and glandular lesions.—*Pathologische Untersuchungen über die diskrete Lungentuberkulose der Erwachsenen, S. Puder, Wien. klin. Wchnschr., September 20, 1928, xxxviii, 1340.*—(*M. P.*)

Types of Tuberculous Ulcers in Inbred Guinea Pigs.—This report concerns the reaction of the tissues, and more particularly the skin, of inbred guinea pigs to inoculation with tubercle bacilli. These animals differed by families in their natural resistance to tuberculosis, and were considered suitable for the determination of some at least of the physiological attributes underlying natural resistance to tuberculosis. The animal material consisted of five families of guinea pigs, which had been inbred by continuous brother-and-sister mating for a long succession of generations. The families are designated by the numbers 35, 2, 32, 13 and 39. They decrease in resistance to tuberculosis in the order stated. On intracutaneous inoculation with tubercle bacilli these inbred guinea pigs develop local lesions having family characteristics. Family 35 (high resistance) develops a compact nodule with a relatively restrained type of ulceration. Family 13 (low resistance) develops a softer, less well demarcated, more oedematous nodule with a much less restrained, more destructive type of lesion. Differences of the same general order develop when local lesions are created on the skin with turpentine or cantharides in these two families. In other words, the distinctions relate to the general quality of inflammatory reactions in these animals rather than to a specific reactivity against the tubercle bacillus or its products. Families 2 and 32 which are intermediate between 13 and 35 in natural resistance develop local lesions which are on the whole intermediate also, but more like those of family 13. However, family 32 reacts somewhat more like family 35, and family 2 more like family 13. Family 39 (lowest resistance) presents a peculiar type of local lesion, which begins with a compact nodule not to be distinguished from that of the most resistant family but often ends with an extensive indolent, excoriating, crusted ulceration. This type of lesion is not always present in this family but never occurs in the other families. In family 13, but in no other, there is a difference in the reaction of the adjacent lymphatics and lymph nodes. Fresh nodules and small ulcers frequently appear along the line of the lymphatics. The lymph nodes often become adherent to the skin, ulcerate through, and present residual persistent sinuses. Families 35 and 13 also present differences in the quality of their tissues suggestive of variations in constitutional character, as observed in connection with a minor surgical operation (intravenous inoculation). In 35 the tissues were stronger; there was less free fat in the areolar tissue and the vein-wall was definitely stouter. This family also had a higher muscular tone than 13 and was more active. The sum-total of the relationships described may be expressed in the following arrangement of family numbers, in order respectively of decreasing general resistance and of decreasing reactive quality. The less definite likenesses are suggested by the brackets. General resistance: [35, 2], [32, 13], 39. Inflammatory reactivity: [35, 32], [2, 13], 39. With reference to the problem of locating reactive characteristics which are both based on the inheritance and are capable of accounting for differences in resistance the agreement is considerable. Although the fact that families 2 and 32 do not follow the scheme suggests that resistance is not wholly determined by inflammatory character, the qualities of lesion exhibited are such as to agree with the general resistance as previously determined, and it is believed that the qualities underlying the tissue reaction may be safely considered to be among the influential factors in the make-up of the natural resistance against tuberculosis. The differences in reaction are in part manifested against agents (cantharides and turpentine) causing simple inflammation also, and hence must be designated as nonspecific with reference to the tubercle bacillus. The accumulated evidence from the study of the separate families and intercrosses between them is to the effect that the differences in question are transmitted by the blending type of inheritance, and are, therefore, controlled by multiple unit characters. It is also suggested by the observations that certain of the significant characters are recessive in nature. It is of considerable interest that the observations agree very well with the older conception of an inherited, predisposing, constitutional diathesis as a

significant factor in the incidence of tuberculosis.—*Ulcerative Types as Determined by Inheritance and as Related to Natural Resistance against Tuberculosis: An Experimental Study on Inbred Guinea Pigs, P. A. Lewis and Dorothy Loomis, J. Exper. Med., March 1, 1928, xlvii, 449.*—(*J. S. W.*)

Late Results of Pulmonary Fibrosis.—The reasons for determining the relationship between the various forms of pulmonary fibrosis are apparent when the possibility of surgical prevention of the graver manifestations of bronchiectasis is considered. Bronchiectasis should not be regarded as a disease, but as the late result of an inflammatory process. There is no early bronchiectasis any more than there is early phthisis with cavitation. Can the occurrence of bronchiectasis be anticipated in chronic interstitial inflammation of the lung? Can any distinctive type of pulmonary infection be recognized as the precursor of bronchiectasis? Chronic pulmonary catarrhs are classified according to the extent of symptoms and physical findings as follows: *1: Chronic Pulmonary Catarrh:* In this group are found cases of obstinate catarrh, usually confined to the lung bases, and with a tendency to recurrence. *2: Pulmonary Fibrosis:* These cases are similar to those of group one, except that the catarrhal signs never disappear. Some permanent pulmonary fibrosis is present. *3: Fibroid Lung:* This group consists of cases of pulmonary fibrosis in which the amount of fibrous tissue is so great that the physical findings are those of solid lung. *4: Bronchiectasis:* In this group the signs and symptoms of bronchial dilatation or basal cavitation are found in addition to the symptoms and signs present in cases of group 3. Does the condition of patients in groups 1 and 2 ultimately become that of patients in groups 3 and 4? In attempting to answer this question the condition of 26 of 167 cases treated from 1905 to 1914, with a diagnosis of "fibroid lung," was determined. The investigation suggests: A diagnosis of postpneumonic fibrosis is only justified when the physical signs of this condition are definite and persist for some months. *2:* When permanent fibrosis results from bronchopneumonia, bronchiectasis is not an inevitable sequel. *3:* Fibroid lung is compatible with fair health over a period of years, provided it is not associated with bronchiectasis. *4:* The study, though incomplete, supports the opinion that the prognosis of bronchiectasis in childhood is always grave, and death before adult life is to be expected.—*The Late Results of Pulmonary Fibrosis, W. B. Wood, Lancet, March 24, 1928, ccxiv, 596.*—(*H. McL. R.*)

Development of Apical Tuberculosis in Adult.—The pathological anatomist does not frequently have the opportunity to observe the early stages of pulmonary tuberculosis unless the patient dies from an intercurrent condition, when the tuberculous foci, according to Zeiss, are usually found in the region of the apical ramus of the upper-lobe bronchus, and in a smaller percentage, in the subapical ramus. Not rarely foci were found in all three dorsal regions of the upper-lobe bronchus, but they were never found in the horizontal ramus, in the absence of involvement of the apical and subapical bronchial regions. The ventral branch of the upper lobe was attacked earliest when lesions or fresh tuberculous processes were found in the three dorsal branches, but never without these. These facts speak in favor of the apical origin of adult tuberculosis. The cicatrix of apical reinfection is that of the so called apical atelectasis. Reinfection, as is to be concluded from the cicatricial reaction resulting from the small number of bacilli and the local protective reaction, develops an intraäcinous productive and not a caseous pneumonic course. As the result of the spread of the process in the bronchial wall, with caseous occlusion of the lumen, there develops a lobular atelectasis, and a further progression of the process leads to a productive intraäcinous dissemination with small foci. Only when the spreading bronchial tuberculosis involves the larger bronchial branches and the amount of disseminated bacillary material is greater, does acinous caseous pneumonia develop. Such large disseminated foci go on either to disintegration and cavity formation, or to nodular encapsulation such as occurs with a primary focus.—*Über Entwicklung, Vernarbung und Reaktivierung der Lungentuberkulose Erwachsener. Zugleich ein kritischer Beitrag zur Lehre von den sogenannten infraclaviculären Frühinfiltraten, H. Loexchcke, Beitr. z. Klin. d. Tuberk., 1928, lxviii, 251.*—(*H. J. C.*)

Primary Tuberculous Cavity.—Although the percentage of primary cavities is given as 36 per cent by the pathologist they are rarely diagnosticated by the clinician. The primary character of the cavity is determined by the state of the regional hilum lymph nodes. The healing of primary cavity as a rule is more rapid than that of a secondary cavity. An interesting case in a 12-year-old girl is reported with roentgenographs.—*Zur Frage der Primärkaverne, K. Klare, Beitr. z. Klin. d. Tuberk., 1928, lxviii, 156.*—(*H. J. C.*)

Effects of Changes in Posture on Chest Volume.—Investigations were carried out on 10 apparently healthy students and 20 cardiac cases. The following change of posture were used: (1) lying supine to standing erect; (2) standing erect to lying supine; (3) lying supine to reclining at 45°; (4) reclining at 45° to lying supine; (5) reclining at 45° to sitting upright; and (6) sitting upright to reclining at 45°. The results of the experiments on normal people show a definite and uniform change with change of posture. On assuming the erect posture there was an excess of inspiration, varying from 493 to 1093 cc., with an average of 822 cc. On assuming the horizontal position there was an excess of expiration from 270 to 903 cc., with an average of 500 cc. *Summary: 1:* With changes in posture of the body the diaphragm alters in level, making a difference in the volume of the chest; this difference in volume is mainly effected by alteration in the volume of the air inspired to the air expired. *2:* A change in level of the diaphragm, on assuming a different posture of the trunk, takes place in less than ten breaths. *3:* In wide changes of posture, from lying supine to standing erect, the difference of volume between inspired and expired air is large. In small changes, the difference in volume is smaller. *4:* The experiments agree with the X-ray findings. *5:* No difference is found in volume between the aortic and mitral cases. *6:* The change in level of the diaphragm might well account for the "postural deflation" occurring in postoperative cases.—*Variations in the Volume of the Chest with Changes of Posture, J. L. Livingstone, Lancet, April 14, 1928, ccxiv, 754.—(H. McL. R.)*

Diaphragmatic Excursions.—Fluoroscopic tracings, taken in both lateral recumbent positions, are of value in recognizing pathological conditions of the lungs, pleura and pericardium. Normally the average excursion of the dependent half of the diaphragm in this series of cases was 6.3 cm., and of the upper half 3.8 cm. The average difference in favor of the lower segment was 2.5 cm. Excursions of the upper leaf smaller than 2.5 cm., of the lower leaf smaller than 4.5 cm., or a difference between movements of the two segments of less than 1.5 cm., are to be considered as pathological. However, when each one of the four measurements in a set exceeds 4.5 cm., the action of the diaphragm is to be considered normal. Normally the brightest illumination of the lungs on inspiration occurs at the base of the dependent lung, and deviation from this occurrence represents lesions in the lung. When the excursion of one segment of the diaphragm is much restricted in both lateral positions, pleural adhesions on the side of the restricted leaf are indicated. When the movements of both sides of the diaphragm are much restricted in one lateral position and are normal in the other lateral position, this restriction indicates abnormality of the lower lung in the position showing the restricted movement. Normally the heart moves away from the dependent chest-wall on deep inspiration, the movement of the lower border of the heart-shadow being greater than that of the upper border. In patients with adherent pericardium this movement of the heart upward on inspiration is restricted or absent.—*Diaphragm Excursions, Normal and Pathologic, P. F. Butler and H. W. Dana, Am. J. M. Sc., October, 1928, clxxvi, 569.—(W. I. W.)*

Pulmonary Ventilation.—The theory that the hydrogen-ion concentration of the arterial blood controls ventilation is probably untenable. There are outstanding instances in which augmented ventilation obtains though the blood is more alkaline than normal. Observations of the author point directly to the importance of the metabolism of the respiratory centre and of the acidity of the centre itself as contrasted with the acidity of the blood. An exception to the rule of control by blood acidity is seen on the injection of sodium bicarbonate. If injection is slow there may be no change. If larger or faster, the blood turns more alkaline and ventilation actually increases. After injection of sodium carbonate ventilation is momentarily stopped. Another exception is the increased ventilation on administration of a gaseous mixture low in oxygen, when both arterial and venous blood turn alkaline. When room air is readministered, the blood turns acid and ventilation subsides. The author has formulated a theory of control to explain the frequent inverse relation between blood acidity and pulmonary ventilation, which is that the respiratory centre possesses an acid metabolism of its own, the acidity being determined by the rate of formation of acid in the centre and the rate of transfer of acid from it. By virtue of its own metabolism and its extreme sensitiveness to minute changes in its own hydrogen-ion concentration, the respiratory centre is sensitive to minute changes in its own oxidations and to changes in the tension of oxygen in the arterial blood. Since the supply of oxygen determines the amounts of lactic acid and carbon dioxide formed in living tissues, and controls the transport and elimination of acid, it constitutes an indirect regulator of pulmonary ventilation. Experiments have been carried out

to follow changes in blood acidity, expired carbon-dioxide, expired oxygen, total carbon-dioxide and lactic-acid content of the blood, volume-flow of blood, mean blood-pressure, pulmonary ventilation, and respiratory movements. Injections of hydrochloric acid and sodium bicarbonate both result in increased respiratory movements, although the effect on all the other factors, with the exception of expired carbon-dioxide, is diametrically opposite. Therefore, the respiratory centre does not discriminate between an acid or an alkaline change in the blood.—*Regulation of Pulmonary Ventilation by Acidity of Blood, Tissue Fluids and Tissue, R. Gesell, J. Am. M. Ass., October 27, 1928, xci, 1256.*—*(G. L. L.)*

Basal Metabolism in Pulmonary Tuberculosis.—Increased basal-metabolism rates are found more frequently in febrile and subfebrile cases of pulmonary tuberculosis than in afebrile cases. An increased basal metabolism is not necessarily associated with fever, but is more frequently an expression of the activity of the tuberculous process. The extent and type of the process are not a measure of the basal metabolism. An unfavorable prognosis cannot be concluded on the basis of an increased metabolism, and vice versa. In the absence of other signs of activity in tuberculosis an increased basal metabolism is always an index for the institution of treatment. A temporary increase in basal metabolism does not need to go hand in hand with an unfavorable progression of the general condition. Since the respiratory quotient in febrile cases of pulmonary tuberculosis is generally lower than in afebrile cases and in those in which the basal metabolism is increased, it diminishes while the basal metabolism increases. A disturbance in the carbohydrate metabolism in pulmonary tuberculosis is assumed to exist.—*Der Grundumsatz der Lungentuberkulösen, Anthony and Kowitz, Beitr. z. Klin. d. Tuberk., 1928, lxviii, 18.*—*(H. J. C.)*

Altitude and Basal Metabolism and Red-Cell Sedimentation.—The basal metabolism, in the same tuberculous individual, on an average, decreased at a high altitude, in Davos, as compared to low altitudes. The milder cases had a greater limitation of the basal metabolism than the severe cases. The red-cell sedimentation rate also diminished at a higher altitude. The voluntary respiratory pause, which is naturall, shortened in a tuberculous individual, was further shortened at high altitudes; on the average, there was a diminution of from 14 to 25 per cent in comparing Basel with Davos. The action of the climatotherapy of tuberculosis at high altitudes is more of a protective than a stimulating nature.—*Vergleichende Untersuchungen im Tiefland und Hochgebirge über das Verhalten von Grundumsatz und Senkungsreaktion bei Tuberkulösen, H. Kapp, Beitr. z. Klin. d. Tuberk., 1928, lxviii, 378.*—*(H. J. C.)*

Influence of Parathyroid Hormone on Formation of Exudates.—The present study was undertaken to determine whether the parathyroid hormone will prevent the formation of experimental effusions. Sixteen experiments were performed on dogs. Seven animals were used for control experiments. The results indicate that the parathyroid hormone (Collip), given subcutaneously, diminishes or prevents pleural effusions following the intrapleural injection of an irritant (solution of copper sulphate). In the dog it appears that considerable hypercalcaemia is necessary, because an animal that had a relatively low serum-calcium behaved practically the same as a normal control animal in response to the irritation. The observation that certain doses of the parathyroid hormone inhibit experimental exudates produced by a given kind and degree of irritation has interesting clinical possibilities. To what extent it will prove applicable for the prevention of clinical effusions remains to be determined by direct observations. Its usefulness will almost certainly be limited by such factors as the cause of the effusion, the intensity of the irritation, the degree to which the hormone will produce hypercalcaemia in man without toxic symptoms, and the degree of hypercalcaemia necessary in man to impair the passage of fluid from the circulation into the tissues or body cavities.—*The Prevention of Experimental Exudates by the Parathyroid Hormone (Collip), H. Gold, Arch. Int. Med., October, 1928, xlii, 576.*—*(W. I. W.)*

Carbohydrates in Tuberculosis.—Although the nitrogenous and mineral exchanges of the tuberculous have been considerably discussed and studied, the metabolism of the carbohydrates, especially sugars, has been comparatively neglected. There are merely scattered observations of alimentary glycosuria or glycolytic insufficiency. In previous studies of protein and fat metabolism Monceaux has shown a retardation of the nutritive processes, due to hypoöxidation, a part of the nitrogenous bodies remaining in the amino-acid stage. Likewise there is a nonutilization of fatty acids. These factors explain the wasting in tuberculosis. A part of these nutritional disturbances appear to be dependent on a hepatic insufficiency, evidenced by numer-

ous tests. Therefore one would expect marked disturbances of carbohydrate metabolism. However, contrary to expectation, carbohydrate metabolism, especially of monosaccharides, has been shown to be practically normal in these studies, which Monceaux details. The chemistry and physiology of the subject is briefly reviewed. It is recalled that under the action of salivary, pancreatic and intestinal ferments and certain colonic bacteria, the carbohydrates ingested are converted into hexoses, which the liver probably transforms to glucose. The complete genesis of glycogen is difficult to arrive at, since certain authors have shown its production by oxidation of proteins and fats. The glycogen reserves of the liver vary with the needs of the organism, whereas the muscle reserves are consumed *in situ*. The intermediate stages of oxidation of glucose are not well known. Attacking the problem in relation to the tuberculous, Monceaux has recourse to blood-sugar variations and the passage of sugars in the urine according to certain tests. Glucose is absorbed chiefly by the blood and lymphatic route and its ingestion immediately increases the liver glycogen. This glycogenic function of the liver is important in alimentary glycosuria. Glucose-tolerance tests in the tuberculous were practically normal. Only 5 per cent of cases showed a glycosuria after 100 gm. of commercial glucose, and this was transitory; with 150 gm., a large dose, 22 per cent showed sugar three hours after ingestion, not an abnormal proportion. A hyperglycaemia normally has been found, however, even after so small a dose as 20 gm., lasting several hours, showing that transformation by the liver does not take place immediately. If the intramuscular or intravenous route of administration is used, about 50 gm. of glucose suffice to produce glycosuria (Achard), while those with glycolytic insufficiency (diabetes, certain acute or chronic infections) show it after as little as 10 gm. Achard found this disturbance constant in cachectic conditions, and frequent in confirmed tuberculosis, especially wasted and febrile patients (45 per cent). This indicates diminished power of fixation of the tissues. Levulose is ingested and utilized similarly to glucose. Normally it is well tolerated and taken in considerable quantities in fruits, certain leguminous vegetables, and as a by-product of saccharose. In these studies it was given to the tuberculous in the form of honey, which is well tolerated and never observed to be followed by levulosuria. Certain patients were given 30 gm. at the end of each meal. It is a dessert which deserves to be more widely utilized. Galactose is not tolerated in as large quantities as glucose and levulose, and alimentary galactosuria occurs after ingestion of smaller amounts. Saccharose and lactose seem to be well utilized, and were never observed in the free state in the urine. Polysaccharides occur especially in the cereal grains, which are of importance to the tuberculous also because of their mineral content, especially oats and beans, rich in calcium, corn, rich in magnesium, lentils in iron, and barley in silicon. In the form of purées, with milk and butter added, these make many very acceptable dishes. The starches are converted by digestion into sugars; as the diastatic ferments are less active in the tuberculous, unchanged starch granules occur in the stools; this is especially true of the legumes, in which the granules are hard and coarse. However, this can be overcome by "maltage" of the cereals, using either the commercial malted cereals, or the malt used to act on "mash." In this way the tuberculous can be given large amounts of amylases which are well tolerated. *Influence of Carbohydrates on General Nutrition:* Recent work has shown their importance in aiding the assimilation of fats, for which reason sweet cakes at the end of the meal are quite desirable. It also appears that sugars may assist in the destruction or conversion of amino-acids, which tend to accumulate in excess from ingested protein and may accelerate the development of the tubercle bacillus. Maillard has determined *in vitro* a reaction between glucose and such amino-acids as glycocol, alanin, valin, leucin, tyrosin, sarcosin, glutamic acid. All the hexoses have this action, especially glucose, and the pentoses. Then why is tuberculosis so severe in diabetics? Because acid intoxication prevents catalysis, as has been clearly shown. Ingestion of sugars appears to have no effect on acetonuria in tuberculous subjects. Nevertheless, the foregoing considerations suggest their wider use, in the form of sugar, honey and confections, and notable gains in weight have occurred with such a régime (up to 100 gm. per day). *Conclusions: 1:* Carbohydrate metabolism in the tuberculous is much nearer that of normal persons than is protein or fat metabolism. *2:* Hepatic insufficiency does not seem to be very important, but tissue insufficiency is marked in the febrile and cachectic. *3:* Carbohydrate rations should be increased in tuberculosis for these reasons: (a) facilitation of fat assimilation; (b) aid in destruction of amino-acids; (c) augmentation of antitoxic power of the liver.—*Les hydrates de carbone dans la nutrition des tuberculeux, R. Monceaux, Rev. d. l. Tuberc., February, 1928, ix, 28.*—(*A. P.*)

Influence of Cholesterol on Experimental Tuberculosis.—It is generally recognized that the high tuberculosis morbidity and mortality rates among the white races are associated with an important, though variable, degree of resistance to the progress of infection. The nature of this resistance is very obscure. A review of the reported chemical analyses of tuberculous tissues shows that the most striking variation from normal is the high value frequently found for cholesterol. Caldwell found that cholesterol increases in tuberculous foci from 200 to 300 per cent, depending on the degree of caseation. Jaffe and Levinson confirmed this, and also found that it was possible to increase further the localized cholesterol by the administration of this substance during the course of the infection. From their experiments they concluded that cholesterol does not protect rabbits against tuberculosis. These experiments were repeated in part by the author with similar results. The accumulation of cholesterol or cholesterol esters in tuberculous tissues, and especially in the actual anatomical tubercle, suggested the possibility of using this substance for chemotherapeutic experiments. It was also thought that it might act as a carrier of radicles known to possess bactericidal properties. In the first series of experiments cholesterol alone was used in treating infected guinea pigs. In the second series various compounds containing cholesterol or an oxidation product of cholesterol were used. Of the compounds used, sodium-cholesterylate and sodium-cholesterol-sulphate were water soluble. The rest were prepared by dissolving in hot alcohol, and pouring the hot alcoholic solution into hot distilled water with constant stirring. The alcohol was removed by distillation *in vacuo*. *Experiment 1:* Twenty guinea pigs were inoculated subcutaneously with 1 cc. of a very thin suspension of recently isolated human tubercle bacilli. They were divided into 2 groups of 10 each. Three weeks after infection the animals in group 1 received 4 intraperitoneal injections of 4 mgm. of cholesterol at intervals of 3 days. The control animals received 2 cc. of distilled water on corresponding days. The average life of the controls was 50 days and the cholesterol treated animals 66 days. *Experiment 2:* Twenty-five guinea pigs received 0.25 cc. of a thin suspension of recently isolated human tubercle bacilli subcutaneously. After 15 days they were divided into 3 groups. Seven controls received 7 injections of 2 cc. of distilled water intraperitoneally every 4 days. Another group of 7 received 7 injections, each of 8.8 mgm. of cholesterol, and the remainder received 7 injections, each of 10 mgm. of irradiated cholesterol, on corresponding days. The average life of the controls was 80 days; cholesterol treated, 113 days; and irradiated cholesterol treated, 103 days. *Experiment 3:* Sixty guinea pigs from family 13 (inbreds) were inoculated subcutaneously with 0.1 mgm. of H7156, human tubercle bacilli. The culture in this amount was known to produce a chronic type of tuberculosis. One group of 30 animals received 10 intraperitoneal injections of 3 cc. of distilled water on the 14th, 18th, 22nd, 26th, 30th, 34th, 38th, 57th, 68th, and 110th days after infection. The other group received 18 mgm. of cholesterol on corresponding days. The average life of the controls was 163 days and in the cholesterol treated group 160 days. *Experiment 4:* Forty-eight guinea pigs (crossbred in heredity) were inoculated with 1.0 mgm. of H7156, subcutaneously. After 5 days they were divided into 3 groups of 16 each. The first group received 10 intraperitoneal injections, each of 3 cc. of distilled water on the 5th, 8th, 12th, 19th, 23rd, 27th, 31st, 103rd, 111th, and 121st days following infection. The second received 10 intraperitoneal injections of Pfanstiehl cholesterol (hog brain), each of 21 mgm. on corresponding days, and the third 21 mgm. of cholesterol, prepared from gall-stones, on corresponding days. The average life of the controls was 71 days; Pfanstiehl cholesterol group, 81 days; and the gall-stone cholesterol group, 76 days. *Experiment 5:* Thirty-two guinea pigs were inoculated with 0.2 mgm. of B14, bovine tubercle bacilli. After 3 days the animals were divided into 2 groups of 16 each. The control group received 5 intraperitoneal injections, each of 3 cc. of distilled water on the 3rd, 6th, 10th, 14th, and 19th days following infection. The other group received 21 mgm. of cholesterol on corresponding days. The average life of the controls was 50 days and of the cholesterol treated 54.6 days. *Experiment 6:* Forty guinea pigs received 1 cc. of a very thin suspension of recently isolated human tubercle bacilli subcutaneously and were divided into 4 groups of 10 each. Three weeks after infection, group 1 received 4 intraperitoneal injections, each of 3 mgm. of cholesteryl-chloride, at intervals of 3 days; group 2, 2 mgm. of cholesteryl-anilide; group 3, 1.2 mgm. of cholesteryl-toluide; and group 4, the controls, 2 cc. of distilled water on corresponding days. The average life of group 1 was 63 days; group 2, 60 days; group 3, 55 days; group 4, 50 days. *Experiment 7:* Fourteen guinea pigs received 0.25 cc. of a very thin, paper-filtered suspension of recently isolated human tubercle bacilli

subcutaneously. They were divided into 2 groups of 7 each. Fifteen days after infection one group received 7 intraperitoneal injections of 10 mgm. of sodium-cholesterol-sulphate at intervals of 4 days. The control group received 2 cc. of distilled water on corresponding days. The average life of controls was 80 days and of the treated group 92 days. *Experiment 8:* Sixty guinea pigs from family 13 were inoculated with 0.01 mgm. of H7156 subcutaneously, and divided into 2 groups of 30 each. The animals in one group received 10 intraperitoneal injections, each of 9 mgm. of quinine-cholesterylate, on the 14th, 18th, 22nd, 26th, 30th, 34th, 38th, 57th, 68th, and 110th days after infection. The control group received 3 cc. of distilled water on corresponding days. The average life of the treated pigs was 168 days and of the controls 163 days. *Experiment 9:* One hundred and five guinea pigs, crossbred in heredity, received 0.2 mgm. of H37 subcutaneously. They were then divided into 7 groups of 15 each. One week after infection, group 1 received 0.1 mgm. of sodium-cholesterylate intraperitoneally twice weekly throughout the experiment. Four groups received 0.5, 1.0, 5.0, and 10 mgm. respectively, on corresponding days. One control group did not receive any injections, and the other had 1 cc. of distilled water on days corresponding to the treated animals. The average life of the untreated controls was 206.9 days and the treated controls 143.6 days; of the group receiving 0.1 mgm. of sodium cholesterylate, 165 days; 0.5 mgm., 189.7 days; 1 mgm., 240 days; 5 mgm., 154.5 days; and 10 mgm., 119.7 days. (Six animals of the last group died as a result of the injections.) Mathematical calculations of the significance of the experiments are presented. *Summary: 1:* Cholesterol, administered intraperitoneally, in these experiments definitely prolonged the lives of tuberculous guinea pigs when the infection was of an acute type produced by inoculation with a small dose of very virulent human microörganisms. *2:* Intraperitoneally administered cholesterol did not definitely prolong the lives of tuberculous guinea pigs when the infection was of the chronic type produced by the injection of a small dose of human tubercle bacilli of relatively low virulence, or when the infection was more acute owing to the injection of a large dose of microörganisms of low virulence. It had no beneficial effect on an acute type of infection produced by the bovine type organism. *3:* Cholesteryl-chloride, cholesteryl-toluide, cholesteryl-anilide, sodium-cholesterol-sulphate and quinine-cholesterylate did not significantly prolong the lives of tuberculous guinea pigs. *4:* Sodium cholesterylate, in optimal dosage, definitely prolonged the lives of tuberculous guinea pigs. *5:* There was a significant shortening in the duration of life of tuberculous guinea pigs subjected to the trauma of intraperitoneal injection and repeated handling, as compared with tuberculous guinea pigs that were not handled or traumatized by intraperitoneal injections.—*The Influence of Cholesterol on Experimental Tuberculosis, R. E. Shope, J. Exper. Med., September, 1928, xlviii, 321.*—(*F. G. P.*)

Serum-Calcium in Experimental Tuberculosis in Rabbits.—Studies were made of the changes in the serum-calcium level in rabbits with experimental bovine tuberculosis, using intraperitoneal and subcutaneous inoculations. After intraperitoneal inoculation the disease becomes generalized, with widespread miliary lesions showing varying extent of caseation. It is more chronic following subcutaneous inoculations. If the animal dies in less than thirty days, the serum-calcium falls in only about one-third of the experiments. When no fall occurs the lesions show relatively little caseation, and in every case in which the fall is considerable caseation is striking.—*The Serum Calcium in Experimental Tuberculosis, J. C. Hoyle, Brit. M. J., September 22, 1928, no. 3533, 523.*—(*W. I. W.*)

Tubercle Bacilli in Organs of Rabbit.—In a recent study by Corper and Lurie of the susceptibility of the various organs of the rabbit to tuberculosis, attention was drawn to the fact that their "native" cellular reaction to the intravenous injection of heat-killed tubercle bacilli varied greatly. While dead bacilli elicit the cellular reaction to their chemical constituents, they do not reproduce all the conditions of infection with living bacilli. In the present article the problem is approached by cultural methods, and an attempt is made to follow the fate of tubercle bacilli in the lung, liver, spleen, kidney and bone-marrow of rabbits infected intravenously with large and small doses of human and bovine bacilli, by determining the number of colonies recoverable from similar quantities of tissue on egg media at varying intervals during the course of infection. The rabbits were killed by a blow on the head, and the organ to be studied removed aseptically. An accurately weighed 1-gm. quantity of each was ground in sand and suspended in a given quantity of fifteenth molar disodium-phosphate. From these suspensions, containing a known weight of a given tissue, certain dilutions were made in the same alkaline medium. One cubic

centimetre of each dilution of each organ was seeded directly on three tubes of Dorset's medium and three tubes of Petroff's medium, carefully divided and spread as evenly as possible. The tubes were then incubated. Another 1-cc. specimen was treated with 3 per cent sodium-hydroxide according to Petroff's method, and likewise seeded. It was found that the ultimate number of colonies did not develop until at least three months had elapsed. Two human and one bovine strain of tubercle bacilli were used. In one series the infecting dose was 0.1 mgm. per rabbit and in another series 0.001 mgm. per kilo body weight. Each series consisted of one group receiving the bovine strain and another group receiving one of the human strains. As a rule the animals were killed two days, one, two and four weeks, and two months after infection. When the number of colonies appearing on the surface of a tube was not more than 200, they could easily be counted directly. When colonies exceeded this number they were estimated by reading certain areas through a square window with the aid of a magnifying glass. Doubtful colonies were smeared and stained. According to the results obtained by cultural methods, the original localization of human or bovine tubercle bacilli in the various organs of the rabbit follows the distribution of particulate matter, being greatest in the spleen, next in the liver, next in the bone-marrow, and least in the kidney. The relative position of the lung among these organs is still doubtful owing to the difficulty of isolating tubercle bacilli in pure culture without treatment at the beginning of infection. It is possibly between the liver and the bone-marrow. The distribution represents the number of colonies obtained from organs one day after infection. The fate of the bacilli thus localized depends largely upon the dosage, whether large or small, and the type, whether human or bovine. The original localization, although a factor, is far from being the controlling factor. At first both types of bacilli grow in all the organs without effective opposition. The human type grows very fast; the bovine type, more slowly. Moreover, the rate of multiplication of both types is very different in the various organs. Thus in the spleen it is much more rapid than in the liver; it may even be negligible in the liver and bone-marrow, while very rapid in the spleen with small enough dosage, especially of bovine bacilli. But, as yet, in all the organs no effective opposition is offered to the multiplication of the tubercle bacillus. Soon a remarkable change takes place, and the liver, spleen and bone-marrow begin to destroy the tubercle bacilli. The time at which this begins depends on the dose and the type of bacillus. With large doses the destruction of human tubercle bacilli becomes apparent between the second and fourth weeks. The destruction of bovine bacilli does not occur until the interval between the fourth and eighth weeks. If small quantities of human bacilli are injected, then destruction in these organs is also delayed until the later interval. By the eighth week after infection with a large dose of human bacilli the destruction is more complete than after a small dose. However, the more rapid growth of the human type is followed by an earlier and more complete destruction of the human type than of the bovine. This change in behavior of the liver, spleen and bone-marrow does not depend on the actual number of bacilli accumulated in the organ. It is probable that the native power of the various organs to destroy tubercle bacilli is insufficient to check their growth effectually, but, as a result of a stimulation most probably associated with the growth of the bacilli, an altered behavior toward them is later brought about so that they are destroyed. However, this destruction is insufficient to eliminate them completely from the spleen, especially after small dosage. The behavior of the tubercle bacilli in the lung and kidney is quite different. Here, with large doses of human bacilli the destruction becomes apparent later, in the interval between the fourth and eighth weeks. With bovine bacilli in similar dosage the bacilli in the lung and kidney continue to increase in number until the death of the animal. With small doses of human bacilli the destruction may not begin in the lung until after the eighth week, when there is almost complete destruction in the liver, spleen and bone-marrow. With the same dosage of bovine bacilli the organisms continue to multiply in the lungs and kidney even four months after infection. It is to be emphasized that the group of organs in which tubercle bacilli are first destroyed is the liver, spleen and bone-marrow, whose common function is to remove foreign particulate matter or bacteria from the body, and of these three the spleen lags behind in its destruction of bacilli. Throughout this study it was seen that the tubercle bacilli, whether human or bovine, and whether in large or small dosage, always grow faster in the spleen than in the liver. This is perhaps connected with the liver having a greater native ability to destroy tubercle bacilli. The acquired ability of the organs to destroy tubercle bacilli becomes increased as a result of the growth of the

bacilli and the accumulation of the products in the body. The acquired ability is then added to the natural ability. It has been shown that the allergic state in tuberculosis as represented by the tuberculin reaction can be brought about more quickly with larger doses of tubercle bacilli than with smaller doses. It is suggested that the virulence of a given type of tubercle bacillus for the rabbit is closely related to the original growth of the bacillus. This is an inverse relation, for the more rapid the growth, the lower is the virulence. The more rapidly a given microörganism grows in the body, the more rapidly is a resistance stimulated and the more rapidly are the bacilli destroyed. On the other hand, the more slowly the microörganism grows, the later this is brought about. Thus in bovine infection, while the bacilli are effectively destroyed in the liver, spleen and bone-marrow, the unabated growth of the bacilli in the lungs and kidney brings about the death of the animal.—*The Fate of Human and Bovine Tubercle Bacilli in Various Organs of the Rabbit, M. B. Lurie, J. Exper. Med., August 1, 1928, xlviii,* 155.—*(J. S. W.)*

Elimination of Tubercle Bacilli.—A previous study of an induced epidemic of spontaneous tuberculosis in guinea pigs showed that the portal of entry was almost always the intestinal tract. It seemed therefore desirable to determine whether the elimination of tubercle bacilli in the faeces is a source of infection, and causes the spread of spontaneous tuberculosis. Though the literature on the elimination of tubercle bacilli by tuberculous patients and cattle is extensive, there is no study which demonstrates that tubercle bacilli are eliminated by the faeces when tuberculous lesions are not in open communication with the surface. In experiment I, five guinea pigs were injected intraperitoneally and one subcutaneously with virulent tubercle bacilli. The faeces of these 6 animals were tested every week by inoculation. Two boluses from each animal were ground up and digested with 3 per cent sodium-hydroxide solution. The suspension was then centrifugated, and the sediment, after neutralization, injected subcutaneously into each of two guinea pigs. All of the animals whose faeces were tested died of generalized tuberculosis. Positive results were obtained at the end of the first week in all of the intraperitoneally infected animals. After this tubercle bacilli were rarely found in the faeces until the fourth week. Tubercle bacilli evidently pass through the tissues of the nonimmune animal, and are eliminated in the faeces during the first week of infection. However, another group of guinea pigs (four) showed only one positive faeces prior to five weeks after infection and that was one day after inoculation. In a third group of 4 infected animals tested, none of the guinea pigs inoculated with faeces taken during the first four weeks of infection developed tuberculosis. Tubercle bacilli were not eliminated until the last few weeks of infection; that is, just before and during the interval when the tuberculin reaction was diminishing in intensity or was negative. To explain the spread of spontaneous tuberculosis it is desirable to determine when tubercle bacilli appear in the bile of infected guinea pigs and to compare the elimination in the bile with that in the faeces. Nine guinea pigs infected subcutaneously were used for this purpose, and these animals were killed at intervals, increasing from one to forty-two days. The gall-bladder in each instance was aseptically aspirated after the abdomen was opened; and the bile obtained, diluted with a small amount of salt, was inoculated into two guinea pigs. In this experiment tubercle bacilli were not found in the bile until the fourteenth day and afterward. In another group of 10 guinea pigs, similarly infected with tubercle bacilli, these microörganisms appeared in the bile of one animal twenty-four hours after infection. The urine aspirated from the bladder of infected guinea pigs did not contain tubercle bacilli until the terminal stage of the infection. Tubercle bacilli inoculated into the tissues of normal animals may enter the blood-stream, and be eliminated in the faeces before immunity against the microörganism is established. With the progress of immunization the bacilli become fixed in the tissues, and do not appear in the faeces. When the infection is widely disseminated, this fixation (resistance) is overcome, and the elimination of tubercle bacilli recurs.—*Experimental Epidemiology of Tuberculosis. The Elimination of Tubercle Bacilli in the Faeces, Bile, and Urine of Infected Guinea Pigs, D. Perla, J. Exper. Med., June 1, 1927, xlv, 1025.—(J. S. W.)*

Tracheobronchial Bacterial Flora.—Cultures were made from the trachea and bronchi of 100 patients who had foreign bodies in the air-passages, to determine what part the bacteria play in this condition. Streptococci were of most frequent occurence, *S. haemolyticus* being less often isolated than *S. viridans* and the nonhaemolytic type. They occurred more frequently when the foreign body was a vegetable or bony one, and in most of such instances the patients were acutely ill. The pneumococcus, mostly of type 4, was obtained in 36 per cent of all

cases. Both *Staphylococcus aureus* and *albus* were frequently isolated, but were not limited to any one type of case, and the length of sojourn of the foreign body did not seem to influence their presence or absence. Occasionally they occurred in pure culture. *Bacillus influenzae* never occurred in pure culture, but was usually associated with *Micrococcus catarrhalis* or *M. tetragenus*. Spirochaetes were recovered only once, and in that case there was a coexisting putrid bronchitis. Yeasts occurred several times, apparently as saprophytes. No particular kind of bacteria could be associated with any one type of foreign body, although the percentage rate of *Streptococcus haemolyticus* was slightly greater in cases of vegetable and bony foreign bodies than in cases of metallic foreign bodies. Although the relation between the type of foreign body, the age of the patient and the resulting symptoms has been emphasized, the bacterial investigation offered very little as a basis for the explanation of the degree of the reaction. The virulence of the bacteria and the resistance of the patient probably play an important rôle. The degree of infection seems to depend more on the type of foreign body, the degree of obstruction to drainage, the age of the patient and the sojourn of the foreign body in the bronchus than on the kind of bacteria present.—*The Relation between the Bacterial Flora and Tracheo-Bronchial Foreign Bodies: A Preliminary Study Based on One Hundred Cases, C. J. Bucher, J. Am. M. Ass., September 1, 1928, xci, 633.*—(*G. L. L.*)

Gram Staining of Acid-Fast Bacilli.—A section, known to contain large numbers of gram-positive microörganisms, failed to show these after having been accidentally left for thirty to forty minutes in 1 per cent sodium-thiosulphate. This led to a study of the effects of sodium-thiosulphate on the gram stain. In the first three experiments a fully gram-positive staphylococcus, a gram-negative bacillus and a gram-positive diphtheroid were used. The technique of the gram stain was as follows: Ammonium-oxalate-crystal-violet, thirty seconds; Lugol's solution, thirty seconds; acetone, to decolorize, five to fifteen seconds; 0.5 per cent saffronine, thirty seconds. The smears were washed with water between each step. These experiments showed that when 1 or 2 per cent sodium-thiosulphate was applied to smears of gram-positive and gram-negative microorganisms at any point in the process of gram staining before the application of iodine, gram staining was not interfered with. When applied after the iodine, both gram-positive and gram-negative germs were decolorized. To determine whether this rule applied also to acid-fast microörganisms, sections containing numerous tubercle bacilli were stained for sixty seconds with crystal-violet, thirty seconds with iodine, decolorized with acetone, and then treated with 2 per cent sodium-thiosulphate. After ninety seconds the bacilli were still well stained but were decolorized after five minutes. In experiment 4 the application of this reaction was further tested. Smears were made of four- to six-weeks-old cultures of all the available acid-fast microörganisms and of twenty-four-hour cultures of 22 gram-positive microörganisms. One smear of each was gram stained by the technique given above, one by the Ziehl-Neelsen method, and one as follows: Crystal violet: acid-fast group, sixty seconds; non-acid-fast group, thirty seconds. Iodine: thirty seconds in each and acetone to decolorize. Two per cent sodium-thiosulphate: acid-fast group, ninety seconds; non-acid-fast group, thirty seconds. Saffronine: thirty seconds in each. An additional smear of each microörganism of the acid-fast group was stained according to the foregoing technique, with a variable length of application of the thiosulphate. In general the ordinary gram-positive microörganisms were decolorized by the thirty seconds' exposure to thiosulphate with the exception of two monilia cultures. These showed incomplete removal of the gram stain, which is probably accounted for by the large size of these yeasts relative to that of ordinary bacteria. The acid-fast group showed a higher proportion of positive microörganisms by the gram thiosulphate procedure than by the Ziehl-Neelsen method, and a still higher one by the gram method. Longer treatment with thiosulphate gave fewer gram-positive microorganisms while shorter treatment gave more. Two cultures, *B. leprae* 362 and *B. tuberculosis* 523 (bovine), were used for more exhaustive tests. Six smears were made of each; one pair was stained by Ziehl-Neelsen technique, and the rest with crystal-violet on the 50°C. hot plate for ninety seconds. One was carried through the usual gram technique as a control; two were treated with Weigert's iodine for thirty seconds, decolorized with acetone and flooded with 2 per cent thiosulphate, one for ninety seconds and the other for seventy minutes; the last smear with thiosulphate only for seventy minutes. All were counterstained with saffronine. The lepra bacillus was not acid-alcohol-fast, but was 70 per cent acid-fast to 5 per cent nitric acid. It was gram-positive, and about 70 per cent positive after thiosulphate treatment. The tubercle-bacillus culture was

positive throughout. A series of smears, from sputum of tuberculous patients, and stained for varying lengths of time on the 50 to 52°C. hot plate with Ziehl's fuchsin and crystal-violet, respectively, showed that at least ninety seconds were necessary to stain tubercle bacilli with fuchsin while thirty seconds sufficed for crystal-violet. No other microörganisms retained the stain when the gram thiosulphate procedure was used. Cold staining with crystal-violet failed to show more than a few bacilli by either the gram or the gram thiosulphate methods. Lepra bacilli were well stained when heat was used in the crystal-violet step. *Conclusions: 1:* When stained by the gram method, the tuberculosis-lepra group resists decolorization with sodium-thiosulphate longer than do other microörganisms. *2:* Non-acid-fast microörganisms, when stained by the gram technique, are promptly decolorized by sodium-thiosulphate. *3:* This differential characteristic applies in sputum and in tissues as well as in smears of cultures. *4:* Apparently, a larger proportion of the tubercle or lepra bacilli present in sputum or tissues may be demonstrated by means of this thiosulphate-resistance technique than by the Ziehl-Neelsen procedure.—*The Gram Stain. II. Resistance of the Tuberculosis Leprosy Group of Organisms to Decolorization with Sodium Thiosulphate, R. D. Lillie, Arch. Path., June, 1928, v, 1044.*—*(F. G. P.)*

Iron and Citrate in Culture Media for Tubercle Bacilli.—Several recent contributions have emphasized the importance of iron in the growth and metabolism of tubercle bacilli. The addition of soluble iron salts to the various protein and synthetic media has been found to be favorable to the growth of the bacilli. More stress has been laid upon the presence or absence of iron than upon its availability to the growing bacilli, which was the principal consideration of this study. The synthetic medium used consisted of asparagin 4 gm., sodium chloride 5 gm., disodium phosphate 2 gm., glycerol 30 gm., and water 1000 cc. Several strains of tubercle bacilli and other acid-fast microörganisms grew rapidly in this medium. The addition of inorganic iron resulted in almost the complete precipitation of the metal. The influence of the pH on the solubility of iron was then studied. Several lots of media were adjusted to give a final pH of from 5.8 to 8.0. Dilute solutions of ferric-chloride were added to give a final concentration of 0.05 gm. per litre. Throughout this pH range the extent of iron-precipitation was approximately equal, a trace of iron always remaining in solution. The precipitates formed in more acid solutions from pH 7.0 upward consisted largely of brown hydroxide of iron. It had been shown that on the addition of sodium-citrate abundant iron was held in solution; 0.1 gm. of sodium-citrate per litre was therefore added to several lots of media. These were adjusted to give a final pH range from 4.6 to 8.6, and sufficient iron was added to make 0.2 gm. per litre. After standing for twenty-four hours the media were filtered and analyzed for iron. The small quantity of citrate added almost entirely inhibited precipitation of the iron, most effectively, however, in the pH range from 6.0 to 8.0. In more acid and more alkaline solutions citrate had relatively little effect on the precipitation of iron. The omission of various constituents of the medium showed that, in the absence of aspargin citrate was slightly less efficient in maintaining iron in solution at pH 7.4. The effect of adding and of maintaining iron in solution on the growth of tubercle bacilli was next studied. Media were prepared as above and divided into three series containing (1) 0.1 gm. sodium-citrate and 0.11 gm. ferric-chloride per litre, (2) 0.11 gm. ferric-chloride and no citrate, and (3) 0.1 gm. sodium-citrate and no iron. Each series was subdivided into five lots and the reaction adjusted to pH 6.0, 6.5, 7.0, 7.4 and 8.0, respectively. The Koch strain of tubercle bacillus was used, and the amount of growth was determined after five and eight weeks' incubation. The microörganisms were autoclaved, washed, dried, and weighed. The results showed that the best growth occurred at pH 7.4. At this reaction and in the absence of iron the tubercle bacillus makes a relatively poor growth. When iron and no citrate was added, there was a slight increase during the first five weeks and approximately 20 per cent increase in eight weeks. When both iron and citrate were added there was a considerable increase in the first five weeks and over 100 per cent increase after eight weeks' incubation. At pH 8.0 there was a similar though slightly less marked increase in growth from the addition of iron and iron and citrate. In the more acid media there appears to be a slight advantage from the addition of iron but very little from the addition of citrate. There was comparatively little decrease in the iron content after eight weeks' growth of tubercle bacilli. This decrease was, however, as great as or greater than the amount of iron soluble in media to which no citrate was added. For comparison *B. leprae* and *B. phlei* were grown on a similar series of synthetic media. The results were similar to those obtained with tubercle bacilli.

The increase in growth associated with the presence of iron was, however, considerably less than in the case of the tubercle bacillus. —*The Action of Iron and Citrate in Synthetic Media for Tubercle Bacilli, G. B. Reed and Christine E. Rice, J. Bacteriol., August, 1928, xvi, 97.*—(*F. G. P.*)

Counting of Tubercle Bacilli.—Methods of counting bacteria are direct and indirect. The indirect include the use of suspensions which are compared with standards by nephelometric, turbidometric and colorimetric means. The standards used for comparison must be determined by some direct counting method. Since tubercle bacilli belong to the pleomorphic and variable species of bacteria, there is a great variation in the appearance and color of cultures from different strains. Could a standard be made for each strain, the method would be theoretically applicable, but impracticable. The volumetric method, in which the relation of the amount of precipitate to the number of bacteria is determined, is theoretically inapplicable for tubercle bacilli because it presupposes that the bacteria are spherical; so Stoke's formula would not apply to tubercle bacilli. The gravimetric method, used by the majority of research men in infection experiments, does not give the actual number of bacteria per unit weight. The number in any given case must be determined by direct means, usually done by stained smears. The variability in size and shape of tubercle bacilli prevents fixing any standard number per unit weight for all cultures. In direct methods the number of bacteria are counted in a given volume of suspension, either on plate cultures (biological), on stained smears (morphological-optical), or in a counting chamber. *Criticism of the Biological Method:* (1) It determines only living bacteria, and Gotschild has shown that the number of microörganisms in a pure culture never corresponds entirely with the number of living microörganisms; (2) not only single microörganisms, but also small clumps may form isolated colonies; (3) not every viable microörganism need form a colony, but only those that find the medium suitable; (4) for these reasons the biological method gives only the minimal number, and even these vary considerably when cultures are made under different conditions and also when the same dilutions are planted. *The Morphological-Optical Method:* Counting the bacteria directly on stained smears, first used for tubercle bacilli in pure culture by Findel (modification of Nutall's method), gives varying results. Findel found 1 mgm. = 35 million bacteria; Klempner, 1 mgm. = 100 million; Selter, 1 mgm. (unit weight) = at least 1 billion; and Calmette, 1 mgm. = 40 million. The latter used bile for a triturating solution. Petroff and Steenken found 1 mgm. (dry weight) = 300 million. The latter dried 1 cc. of the filtrate of a suspension made with NaCl pH 7.8 and calculated the number of tubercle bacilli per unit of dry weight. *Criticism of All Stained-Smear Methods:* (1) Impossible to get even distribution of smear; (2) results not consistent with each other, nor with other methods; (3) bacteria may be washed from slide during preparation of smear; (4) cells become superimposed on bacteria and so obliterate some of them. The use of the counting chamber, first introduced for tubercle bacilli by Jennings, made determinations of the number of bacteria in a suspension more accurate, provided the suspension is homogeneous and free of clumps. This is the chief difficulty with all direct methods. Selter found that overnight sedimentation still left clumps behind. Only after marked centrifugation were these thrown down, so that single microörganisms remained in the supernatant liquid. Clumps arise not only during the process of growth, but also, as shown by Bruno Lange, by the use of NaCl as a triturating solution. Filtration, as carried out by Petroff and Steenken, and Jennings, provided a suspension free from clumps, but the triturating solution used by them did not fulfill the necessary requirements to obtain a homogeneous suspension. The authors use N/15 sodium-oxalate solution with pH 8.7. The experimental work which led up to this is discussed in detail. To summarize, this solution fulfills the two important postulates: (1) Suitable pH of the solution lowering the surface tension to facilitate frothing during trituration. The optimal pH between 8 and 9, at which the least spontaneous agglutination of bacilli occurs, had previously been worked out by Jennings. (2) By the formation of insoluble calcium oxalate the "agglutinating substance" is removed, and so allows the bacteria to loosen up from their growth-clumps. The suggested method obviates the use of a filter and obtains single microorganisms by the addition of an oxalate which is supposed to precipitate the calcium that acts as an agglutinating substance during the process of growth, and is, therefore, responsible for clumps. His reason for the latter is based on experimental work of Herbst who demonstrated that a microorganism in the gastrulation stage, when brought into a calcium-free medium, falls apart into single cells, whereas on the addition of calcium normal development with agglutination of cells occurs. A

similar calcium-pectin combination is assumed in the growth process of tubercle bacilli, resulting in clumps. To prepare the sodium oxalate, neutral sodium oxalate was employed, a N/15 solution prepared, and brought up to the desired pH 8.5–8.7, by addition of soda. *Technique of Counting: Preparation of Material:* To ensure the exclusion of all foreign particles, thorough cleansing of all apparatus is done. Bürker's counting chamber is employed with the following specifications: chamber height 4.5 mm., chamber depth 0.02 mm., length of one side of square 1/40 mm. The chamber is cleaned with chromic acid for one-half hour, washed with redistilled water, washed with 70 per cent alcohol, and finally soaked in ether-alcohol (1:1) ten minutes. *Trituration of the Tubercle Bacilli:* A portion of growth from any solid medium is weighed out to 10 mgm. on a watch-glass and then triturated to a pasty consistency for one minute in an agate mortar. Then three drops of the sodium-oxalate solution are added, one by one, at one-minute intervals with gentle trituration, then ten drops twice, and finally three drops. Ten drops having been added, then the foaming emulsion is taken up with a pipette and transferred into a prepared reagent glass. To the residue in the mortar, five drops are added twice, and finally ten drops, the trituration being continued. The emulsion is made up to 10 cc. with sodium oxalate; after thorough mixing the final suspension is made by diluting 1:10 so that 1 cc. = 0.1 mgm. *Optical Conditions for Counting:* Working in a dark field, with a magnification of 400, will enable one to differentiate the bacilli from calcium-oxalate crystals. The latter are recognized by their shape, greater refraction of light and slower speed of motion. *Results:* 50 squares are counted. The formula for calculation is as follows:

Length of square $a = 0.025$ mm.

Depth of Chamber $t = 0.02$ mm.

Average number of bacteria in 1 square $x = 3$.

The number of bacteria in 1 cc. of used suspension is:

$$X = \frac{1000\,X}{a^2t}$$

This is multiplied by 10 to get number in original suspension or 1 mgm. The authors give a table showing the variation in the counts, depending upon the age of the culture, the number of generations, and the medium used for growth. The bacterial count in 1 mgm. of tubercle bacilli varied from 1.7 to 3.6 billion.—*Betrachtungen und Versuche zur Bestimmung der Keimzahl in Tuberkelbazillenkulturen, W. Blumenberg and H. Wiening, Ztschr. f. Tuberk., 1928, li, 417.—(P. A. G.)*

Filterability of Tubercle Bacillus.—Fontes made the following observation in 1910: Berkefeld filtrates, prepared from tuberculous pus and inoculated into guinea pigs, gave rise to induration and hypertrophy of lymph nodes. At necropsy the animals presented an atypical picture not comparable to that of tuberculosis. Typical acid-fast microörganisms were found in the lymph nodes, which, after injection again into guinea pigs, produced no pathological changes. Interest in this observation has been renewed by intensive study, chiefly in France, in connection with the Chamberland filter-passing form of tubercle bacillus, with the object of settling the question of congenital tuberculosis. A number of investigators obtained results similar to that of Fontes. Some of these were also able to cultivate the filtrates of tubercle-bacillus suspensions and filtrates of diluted tuberculous materials. A number of others were unable to detect any filterable forms of tubercle bacilli. These studies have paved the way for investigators of hereditary congenital tuberculosis. Up to the present, the experimental and clinical data have shown that tubercle bacilli are transmitted very rarely from mother to offspring, when the placenta is perfectly normal. The method used in the following studies, with the exception of a few details, was essentially similar to that employed by several previous workers. Great care was taken to eliminate any possibility of contamination with acid-fast microörganisms. The apparatus used was cleansed with either nitric or chromic acid. Berkefeld candles, types V, N and W, were cleansed with 0.5 per cent potassium permanganate followed with sodium bisulphite, and washed with 1000 cc. of distilled water, and all were sterilized at 15 pounds pressure for one-half hour. Sixty-five-day cultures of tubercle bacillus H37 in potato-broth were filtered, first, through paper, and then through a Berkefeld filter V, applying a vacuum of 22 inches. One-half of this filtrate (250 cc.) was again filtered through candle W. To 200 cc. of the filtrates 0.5 cc. of 5 per cent freshly prepared tannic acid was added. They were then centrifugated, the precipitate dissolved in 2 cc. of 0.85 per cent sodium chloride, and a small portion of it inoculated on several slants of egg medium. Four guinea pigs were inoculated intratesticularly, each with 0.25 cc. of the sediment. Smears were also stained with Cooper's (modified Ziehl-Neelsen) stain and occasional typical acid-fast granules could be seen. None of the four guinea pigs, after the 202nd day, developed any trace of tuberculosis with exception of acid-fast rods in lymph nodes in animals 2, 3, and 4. Several intracutaneous tests with tuberculin

were negative. A synthetic-medium culture was treated in approximately the same way. Filtrates of H37, of various ages ranging from three or four weeks to several months, were mixed and 5 cc. injected into guinea pigs 5, 6, and 7. The animals were killed on the 186th and 187th days. No macroscopic evidence of tuberculosis could be found at necropsy. Smears from the lymph nodes showed a few typical acid-fast rods in two of these animals. The intracutaneous reaction proved repeatedly to be atypical. Many investigators have pointed out that a medium of low surface tension may facilitate the passage of microörganisms. In a few instances sodium ricinoleate was employed with the hope that, by changing the surface tension, and the soap acting as a lubricant, the tubercle bacilli might pass through the Berkefeld filter W. The tannic acid precipitate, from filtrates prepared by this method, was inoculated into guinea pig 8. Smears of the precipitate showed typical acid-fast granules and one showed one typical acid-fast rod. The animal was killed on the 150th day. No macroscopic or microscopic evidence of tuberculosis could be found except from the pool of the lymph nodes in which 5 typical acid-fast microörganisms were found. An attempt was made to repeat the experiment of Valtis and Calmette with the filtrates of autolyzed positive sputum. Three-day specimens of sputum were diluted with 9 parts of 0.85 per cent sodium chloride and incubated at 37.5°C. for seventy-two hours. They were then filtered through various Berkefeld candles, V, N, and W, and 9 to 10 cc. inoculated into each of 10 guinea pigs, numbers 9 to 18. One of these animals died on the 156th day of generalized tuberculosis and one on the 82nd day of intercurrent disease with no evidence of macroscopic tuberculosis. Typical acid-fast microörganisms were demonstrated in smears of the latter after a long search. A portion of this material was inoculated into two guinea pigs but failed to infect after one hundred and fifteen days. The remaining animals were killed between one hundred and seventy-nine and one hundred and eighty-seven days. No macroscopic evidence of tuberculosis was found, although atypical skin reactions were demonstrated from two weeks after inoculation up to the time of death. The smears of the nodes showed typical acid-fast microörganisms in four and in the others atypical forms. All cultures of sputum-filtrates were negative. Duplicate smears of tannic-acid precipitates of filtrates from sputa (Gaffky V, VI, and VII) were made, and searched for one and one-half hours. One smear from Berkefeld-candle-N filtrate showed two typical acid-fast bacilli, while all of the others were negative. Cultures on plain egg and gentian-violet were negative after four months. Pus from lymph nodes of an animal inoculated intratesticularly with tubercle bacilli was treated in the same way as the sputum and inoculated subcutaneously into two guinea pigs. These animals lived one hundred and ten and one hundred and ninety-two days, respectively, and showed no macroscopic evidence of tuberculosis. Typical acid-fast microorganisms were demonstrated in but two instances in filtrates of positive sputum. One must always keep in mind that imperfect filters may allow intact microörganisms to pass through. The transmissibility of filterable forms of tubercle bacilli from mother to offspring was studied with 16 young, which were born from seven of the animals used in the preceding experiments. Some of these young guinea pigs were killed at birth, others after one hundred and twenty-six days. The tuberculin test was uniformly negative. In 12 out of 16 acid-fast granules were demonstrated in smears of the lymph nodes, but no typical acid-fast rods. A number of animals from a new shipment, dying from intercurrent diseases, offered an opportunity to study the question of acid-fast granules in apparently normal guinea pigs not reacting to 0.2 cc. of 10 per cent old tuberculin. Typical acid-fast granules were demonstrated in 5 out of 15 animals examined. Cultures of these microörganisms were negative. These findings throw a new light on the methods used in examining diagnostic animals. More importance should be placed on the intracutaneous tuberculin reaction than on finding acid-fast bacilli, especially in the absence of definite lesions. The mass of experimental data on the subject is very confusing. The positive results reported have been based chiefly on the presence of acid-fast bodies in the lymph nodes of the inoculated animals. As has been pointed out, acid-fast microorganisms were demonstrated in 33 per cent of normal guinea pigs and in 36 per cent of those inoculated with tuberculous filtrates, excluding the two animals with a definite visceral lesion. Could it not be possible that some microörganism like the timothy bacillus, which is present in hay, the main food of the animal, may enter the body of the guinea pig through the mouth and be entrapped in the lymph nodes? Furthermore, in the previous studies reported in which the intracutaneous test was done, no mention was made as to the type of the reaction. In the present studies no reaction was considered positive without a definite induration of at least 8 to 10 mm., the erythema not being

pale but a deep red, with probably a small whitish centre and better still a necrotic centre. Much stress has been laid upon the cachexia which is so commonly present after the inoculation of filtrates. It is not an uncommon observation that inoculation of large amounts of foreign protein, either peritoneally or subcutaneously, may cause a cachetic state in animals. Intercurrent diseases are also very often responsible for the cachetic state. As to the transplacental passage of a filterable form of the tubercle bacillus, the evidence is not sufficient to uphold the theory that such a passage is due to special filterable forms of the organisms. The cases reported by Calmette, Valtis, and Lacomme, in which positive results were obtained from stillborn infants, are not necessarily due to a filter-passing microörganism. Debré and Lelong, studying 433 cases similar to those mentioned above, did not find any trace of tuberculosis in a single infant or stillborn baby. Formerly it was thought that placental tuberculosis was a rare occurrence, but a number of authors have pointed out that it may be present in unsuspected individuals. In clinically active tuberculosis, in all probability the permeability of the blood-vessel wall may be considerably altered, and the intact microörganisms may escape into the foetal blood circulation without leaving a trace. If the placenta is tuberculous, the microörganism may more readily enter the circulation of the foetus. *Summary:* No growth was obtained from Berkefeld filtrates prepared from pure cultures of tubercle bacilli or from sputa which contain large numbers of the microörganism. No demonstrable tuberculosis, leading to progressive disease, was observed in either the mothers inoculated with such filtrates or in the offspring. Acid-fast granules were demonstrated in such offspring, and in about 36 per cent of the full-grown guinea pigs which had received filtrates. However, these acid-fast microörganisms were present in 33 per cent of apparently normal animals which had never been inoculated. The positive results reported by others in all probability may be due to microörganisms which occasionally can pass through the Berkefeld filter. Several factors may play a part in such passage, as follows: Imperfect filters, the pressure used for such filtration, the medium employed, and the electro-charge of the candle.—*"Filtrable Forms" of the Tubercle Bacillus, F. B. Cooper and S. A. Petroff, J. Infect. Dis., September, 1928, xliii, 200.—(F. G. P.)*

Filterability of Tubercle Bacillus.—Up to the present, pathogenic microörganisms have been classified into two principal categories: (1) microscopically visible elements, whether easily cultivable or not, and (2) invisible elements, despite highly perfected instruments, yet proved to exist and confer disease on man and animals in series. There are, however, microbic elements, visible and cultivable, but capable of traversing masses of porous porcelain or infusorial earth, which make a third and new category. *1: Filterability of Tubercle Bacilli:* In 1907 Much noted acid-fast granular forms capable of metamorphosing into typical bacilli. Bacteriologists had long been puzzled by their failure to find the microörganisms in tuberculous pus, although typical lesions developed in inoculated animals. Then in 1910 Fontes conceived the idea of filtering this pus in order to separate the bacillary from the granular forms. He used physiological salt solution to dilute the material from a caseous lymph node, and passed it through a Berkefeld filter. Microscopic examination of this material was negative. Another portion of the filtrate was inoculated subcutaneously into a guinea pig. After fifteen days a slight lymphatic swelling appeared, and the animal was killed. Smears from organs showed no tubercle bacilli, but part of the spleen, when reinoculated, killed another animal in five months and acid-fast bacilli were found in the lungs and inguinal lymph nodes. Fontes concluded that Much's granulation might traverse Berkefeld filters and produce atypical tuberculosis in animals. Philibert repeated these experiments in 1912, using 10 specimens of tuberculous pus from different sources. Tubercle bacilli were present on direct examination in one case, and on homogenization in five. Philibert reached negative conclusions as to filterability, but he stated that the filtration of the pus was very slow, lasting eighteen days, and that reinoculation of the organs of the first animal was not done. About this time Heymans published a work on the permeability of membranes to microbes. He stained with Gram's stain the reed-membranes which had allowed anthrax bacilli and streptococci to pass through, and determined the presence of abnormal forms, chains of smaller and smaller granules. He believed that plasmodic and ultradiapedetic movements, together with multiplication and reduction in size, force the microorganisms through the pores. In 1922 Vaudremer used potato-water cultures of tubercle bacilli and discovered various atypical forms, especially of filamentous and granular type. Filtering his cultures through a Chamberland filter and planting them on Petroff's media he could obtain

acid-fast or non-acid-fast forms, according to the number of replants, on poorly nutritive media. He ended with cultures cultivable on plain broth, and producing local indurations on subcutaneous inoculation. In 1923 Valtis examined tuberculous sputum for filterable elements. He warmed this to 38° C. to produce autolysis and facilitate filtration. Cultures of the filtrates remained sterile, but subcutaneous animal inoculation resulted positively. The regional lymph nodes enlarged slightly from the tenth to the fifteenth day and then returned to normal. Necropsy of these animals, most of which died between the third and fourth month, showed swollen tracheo-bronchial nodes and pulmonary hepatization, containing characteristic acid-fast bacilli. Analogous results were obtained with the pus filtered from the caseous mesenteric lymph node of an ape dead of tuberculosis. Durand and Vaudremer, as well as Arloing, Dufourt and Malartre, obtained similar results with pus from other sources. Cultures, however, remained sterile, except for the first ones of Vaudremer and Durand. The types of lesions produced by the filtrates are of interest. Durand and Charchansky's findings were as follows: *1:* Forms characterized by tuberculization with absence of "chancre of inoculation" and visceral lesions, and without marked macroscopic lymphatic changes but with tubercle bacilli present in the lymph nodes. *2:* Forms characterized by classical nodular and caseous lesions. Arloing and Dufourt add a third form, consisting of an infection of short duration, without effect on the general state, curing spontaneously with destruction of the infecting microörganism and only transitorily giving an intracutaneous tuberculin reaction. *Direct Transmission of Tuberculosis from Mother to Child:* Calmette and coworkers reported, in 1925, experimental proof that the subcutaneous injection of filtered tuberculous peritoneal exudate into pregnant guinea pigs caused in the young lymphatic lesions, especially of the subhepatic nodes, which contained typical acid-fast bacilli. These results were confirmed by Arloing and Dufourt with filtrate of caseous tracheobronchial lymph nodes. In 1926 the latter brought definite proof of hereditary human transplacental infection in a child prematurely born at seven months of a mother with advanced pulmonary tuberculosis. Although separated at birth it died two months later. Autopsy revealed nothing but enlarged mesenteric lymph nodes, from which, however, tubercle bacilli were recovered. The intrauterine transmission of tuberculous virus from mother to child was recently strikingly shown by Calmette, Valtis and Lacomme. Of 21 premature children or foetuses in the tuberculosis service of Baudeloque's obstetrical clinic, ten came to necropsy. Three showed tubercle bacilli on direct examination, while all had in their viscera and the coronary and mesenteric lymph nodes virulent elements which on guinea-pig inoculation yielded almost constantly positive results. In one case the only visible lesion was a slight tracheobronchial adenopathy containing abundant bacilli. *2: Filterability of Other Organisms:* Work done with the following microörganisms is cited: Avian tubercle bacilli, paratubercle bacilli, *B. typhosus*, enterococci and staphylococci, streptococci, influenza, acute coryza, *B. pestis*, *B. diphtheriae*, *B. typhi exanthematici*, mumps, rabies, poliomyelitis virus, and others. The existence of filterable forms of numerous microörganisms being regarded as proved, the cause of these transmutations becomes a prime question. The dysentery and typhoid bacilli, staphylococcus and others, only acquire their special state under the influence of a bacteriophage, which exerts an autolytic action. During this bacteriolysis these forms appear, as shown by d'Hérelle. It should be understood in all this work that the integrity and sterility of the filters were carefully controlled. *Deductions:* For the filterable forms of tubercle bacilli Calmette, Valtis and Lacomme propose the term *Tuberculous ultravirus*, while Burnet prefers *inframicrobes* and *arthromicrobes*. Nicolle uses the term *infravisible microbes*. All these findings are of interest and importance in many fields and much further investigation is necessary. —*État actuel de la question de la filtrabilité microbienne, F. de Potter, Rev. Belge d. l. Tuberc., November–December, 1927, xviii, 266.*—(*A. P.*)

Filterability of Tubercle Bacillus.—Guinea pigs were inoculated subcutaneously (and occasionally intraperitoneally) with filtrates of old and young cultures of virulent tubercle bacilli, and of positive sputum, pus, urine, stools, and organs from tuberculous men and animals. The material was passed through either Tsigmondy-Bachmann membrane-filters or Chamberlain-Keizer filters, which were tested by filtration of *B. coli* and *B. prodigiosus* and several nonpathogenic and cold-blooded acid-fast bacilli. The filtrates were inoculated in amounts of 2 to 5 cc. into each of 51 guinea pigs. Unfiltered material was inoculated into 9 guinea pigs as controls. The latter were all dead of tuberculosis within eight weeks of the inoculation. Of the 51 that received the filtrates 7 died of intercurrent disease; 5

(inoculated with filtrates through membrane-filter) died one to four months later with unquestionable tuberculosis, some of them having cavities of the lungs and numerous tubercle bacilli demonstrable in the tissues; 9 died of cachexia with slight enlargement of the lymph nodes, slight hepatization of the lungs, and an occasional small mass in the liver which resembled tubercle, but which proved, on inoculation into other animals, not to be tuberculous. The 30 remaining guinea pigs which had received injections of filtrates were killed at intervals of from two and a half to six months after injection. The organs and tissues of none of these showed acid-fast bacilli, and all proved to be sterile when planted on culture, while inoculation into new, normal guinea pigs produced no tuberculosis. But some of the latter animals developed sensitiveness to tuberculin one month after injection which lasted a short time. It may be supposed, therefore, that these animals had been infected and had survived a slight amount of disease. The fact that all the "takes" followed injection of filtrates from membrane-filters may be important, and it is possible that small cracks existed in these. Experiments have shown the transmissibility through the placenta of a filterable virus of the tubercle bacillus. Of 33 young guinea pigs, carried during the time filtrates were injected into the mothers, none showed any signs of tuberculosis. Two yielded acid-fast bacteria on smears, but none on culture or inoculation of other animals. Young rabbits of a gravid mother, which was injected with filtrates four days before she littered, revealed no signs of tuberculosis. However, organs of these young, when injected into 3 guinea pigs, produced great emaciation and some enlargement of lymphoid tissue in two. The nodes from these, when injected into other normal guinea pigs, produced enlargements of the lymph nodes in which acid-fast bacteria were proved microscopically. These inguinal nodes, introduced into another lot of normal animals, produced true tuberculosis. The liver and spleen from these guinea pigs failed to cause tuberculosis, however, when injected into other guinea pigs. The conclusion seems obvious that the tubercle bacillus, so well known as an acid-fast rod, has an invisible, filterable form which may occasionally pass through the placenta and develop into an acid-fast microörganism in the foetus. These bacteria appear to be of low virulence, and but seldom produce progressive tuberculosis. Whether their stay in the body is in any way related to immunity to tuberculosis cannot be answered at this time.—*Uebertragung der Tuberkulose durch filtrierbare Erreger, Lydia Rabinowitsch-Kempner, Deutsche med Wchnschr., November 18, 1927, liii, 1982.*—(*H. S. W.*)

Properties of Tuberculin.—The various tuberculins elaborated since Koch differ not only in composition but in reactive properties. The diversity of their effects is such that scientific opinion is endeavoring to formulate a precise and uniform method of titration. Koch's original method, based on toxicity for tuberculous guinea pigs, is too uncertain, despite Otto's improvements. In 1924 the Hygienic Section of the League of Nations placed on its program the study of tuberculin and methods of titration, and charged Calmette to investigate the technique of preparation and titration employed in different countries. The results were given in a report of April, 1926, to the same section. *In vitro* we are limited to the study of two reactions, precipitation and complement fixation; *in vivo* to the cutireaction in the tuberculized subject and the intracutaneous reaction in the tuberculous guinea pig. *Tuberculin Reactions in Vitro and in Vivo: A: Nature of Active Principles of Tuberculin:* Generally speaking, the name "tuberculin" is applied to all the toxic products obtained by culture of tubercle bacilli, including both exotoxins and endotoxins. After the discovery of Old Tuberculin, efforts were made to improve and purify the product, by precipitation, filtration, emulsification of bacilli, cultivation on synthetic media, etc., in order to eliminate foreign reacting substances. Tuberculin differs from ordinary bacterial toxins in being scarcely affected by heat, other physical agents, addition of antiserum, etc. Early studies of tuberculin, purified by alcoholic precipitation, showed that it gave all the protein reactions, but it was classified as a polypeptide rather than a complete protein. Zinsser puts it in this category. According to Much there is a water-soluble fraction, also albuminous residue, lipoids, fats, fatty acids and alcohols. Long and Seibert consider the active principle of protein nature because its activity is destroyed by trypsin. There is, however, according to Laidlaw and Dudley, an associated precipitating substance resistant to trypsic digestion and thermostabile. This precipitating substance, free from protein, is active with serum in a dilution of 1 to 6,000,000. It is of resinous nature and reacts characteristically to immune serum. This substance appeared to have no antigenic power and gave no intracutaneous reaction in tuberculous guinea pigs. *B: Antigenic Properties of Tuberculin: Methods of Titration:* To prepare an antituberculinic serum, bacilli

killed by heat are generally used. Calmette's biliated bacilli may also be used. Horses are injected, first twice, and then once weekly, with increasing doses of bacilli. The antibody content of their serum rapidly increases but does not persist long. Complement deviation may be used for the titration of tuberculins. Calmette and Massol's method, mixing 0.1 cc. of serum with a 1–20 dilution of tuberculin, was used, and the number of antigen units per cubic centimeter was expressed numerically. The Pasteur Institute standard tuberculin thus evaluated had a strength of 130 units. Twenty-three tuberculins, thus titrated, varied in strength from 0 to 200 units. The activity of the human tuberculins thus titrated varied between 55 and 115 units, the bovine between 40 and 200 units. One flask of *fleolin* (from the dung or timothy bacillus) gave a titre of 130 units. Tuberculins prepared from Sauton's medium were inactive; those from glycerine-bouillon varied from 60 to 200 units. There is no precise correspondence between toxicity and activity as measured by this titration process. *Fleolin*, for instance, is absolutely nontoxic. The precipitation reaction has also been used to detect tuberculin. To obtain a precipitating serum Dreyer and Vollum inoculated horses intravenously with increasing doses of their "diaplyte vaccine" (emulsion of defatted bacilli by acetone and formalin). Watson and Heath also used their method. They found the strongest precipitation to occur with bovine tuberculins, tuberculins free from albumin, and mixed tuberculins. Human tuberculin varies considerably in its precipitating activity. On the whole there appears to be no relation between complement-fixing power and precipitating activity. *C: Reacting Properties of Tuberculins and Methods Derived:* The term "allergy," as Pirquet applied it, means a new reacting aptitude shown by the tuberculized animal and differing from that of the normal animal. These specific allergic reactions vary according to the mode and place of application of the tuberculin. They may be local, general or focal. In studying the activity of tuberculins the intracutaneous Pirquet reactions have been used. Petroff and Stewart, and Boquet, Négre and others showed that cutaneous hypersensitiveness could be produced with dead bacilli, usually appearing ten to fifteen days after inoculation, but Zinsser and Petroff showed that this sensitiveness is relatively transitory. Boquet and Négre also demonstrated a general thermic reaction to tuberculin after the eighteenth day following intraperitoneal inoculation. Also, Calmette, Guérin and coworkers claim that an avirulent strain can produce hypersensitiveness without tubercle formation. Tuberculin itself, however, seems unable to produce hypersensitiveness (Calmette's "pseudoreactions" appeared to be due to impurities in the tuberculin). *Mechanism of Tuberculin Reactions:* Koch assumed that tuberculin activates a process of necrosis in tuberculous foci. Wassermann and Bruck in 1906 proposed an elaborate interpretation based on the supposition of an "antituberculin." Bail believed there was an agressin or a special secretion of the bacillus produced by contact with animal tissues. Maurice Nicolle explained the reactions as depending on a lysin of tuberculous endotoxin existing in the humors of tuberculous subjects; under its action the injected tuberculin would decompose and set free a toxic substance. Wolff Eisner adopted a similar theory, and the theory of lytic actions has now been rather generally adopted. *Appearance and Manifestations of Hypersensitiveness:* A variable time elapses between the moment of infection and the appearance of the specific reaction to tuberculin. Krause found hypersensitiveness to commence with the beginning of infection and the development of the initial focus, increasing with the extent of the disease and varying with the degree and intensity of the latter to end when it is cured. However, it is not entirely lost as soon as healing occurs and may again increase with a reinfection. On the other hand, hypersensitiveness disappears during a general tuberculin reaction. The opinion that there may be some relation between hypersensitiveness and immunity has been expressed by Krause, and by Debré, Paraf and Dautrebande, and others. The latter workers found that the anteallergic period varies inversely with the size of the infection. Hypersensitiveness, they found, does not appear until the primary nodule of infection becomes perceptible to the finger. It persists as long as there is an actively growing bacilliferous lesion. It may disappear in the terminal stages of the disease or during some intercurrent diseases, (influenza, measles, etc.). It is thus possible, by careful measurement of dosage, to provoke as much or as little hypersensitiveness as desired, and in a series of animals of the same age and size, similarly infected, it will appear at practically the same time. Pirquet's observations on the cutireaction were based on prior studies with smallpox vaccine. Working with this, he noticed, in persons previously effectively vaccinated, that a premature papular reaction, even within twenty-four hours, was the rule. The papule, which appears in the skin of tubercu-

lous children after injection or scarification with tuberculin, closely resembles this other and possesses considerable diagnostic value in the early years of life. It is negative in the earliest phase of infection, and in miliary tuberculosis, tuberculous meningitis, and the terminal stages of tuberculosis. In adults of most civilized countries, especially in cities, it is of little diagnostic value. In the present studies tests of guinea pigs were considered less reliable than those of human beings, which were selected from Bernard's service at the Hôpital Laennec. The standard tuberculin for comparison was a mixture of human and bovine tuberculins in proportion of 1 to 3, from the Pasteur Institute. Scarification tests were done, and read after 24 and 48 hours. Four lots of mixed and human tuberculins gave a reaction equal to the standard. One of the bovine tuberculins showed little activity and that of albumin-free tuberculins was variable. Avian tuberculins showed little activity, and bouillon, *fleolin* and mallein were always negative. The intracutaneous reaction is more sensitive and has certain other advantages over the Pirquet reaction. However, it is not regarded as entirely innocuous. Crude tuberculin gave more numerous and intense reactions than any of its protein constituents, except in three cases. The sensitiveness of tuberculous subjects to foreign protein of any kind was confirmed in a number of cases. B. and E. Lange's contentions, however, that extracts of other acid-fast bacilli yielded stronger reactions than tuberculin, were not confirmed. The reactions to peptonized water and *fleolin* are generally precocious, disappearing after twenty-four hours, though some redness may persist. There was considerable difference between different samples of tuberculin. In order to check up reacting specificity, these were also tested on tuberculous guinea pigs, as were certain nonspecific products. All the animals reacted uniformly to 0.1 cc. of a 5 per cent dilution of Pasteur Institute tuberculin. Of the related products tested, *fleolin* was most active. Forty days after infection *fleolin* produced quite intense reactions. Of 8 normal guinea pigs tested with both tuberculin and related products none reacted positively. *Sensitiveness of Animals Inoculated with Paratubercle Bacilli to Tuberculin and Nonspecific Product:* The bacillus used to inoculate was the dung or hay bacillus, 1 to 2 cgm. being given at five-day intervals. The intracutaneous tests were made twelve days after the last injection, using concentrated bouillon, bouillon without peptone, peptone water and *fleolin*, also tuberculin. *Fleolin* in each case provoked more marked reactions than tuberculin, and a second test after twenty-five days showed an accentuated reaction. There is also some aptitude toward reaction with avian and bovine tuberculin, feeble in the former case. *Conclusions:* In the tuberculous human being, tuberculin allergy, characterized by a positive intracutaneous reaction, is accompanied by a hypersensitiveness to nonspecific products, less marked for *fleolin* and trichophytin than for peptone-broth, bouillon without peptone, and peptone water. With these substances skin reactions may be obtained often in as high dilutions as with tuberculin, but they appear a little earlier and are of shorter duration. On the other hand, in the guinea pig the intracutaneous reactions are more differentiated, and tuberculous animals react to tuberculin in much higher dilutions than for the other substances. The normal guinea pig reacts scarcely at all to any of these products. Infection with a paratubercle bacillus induces a marked sensitiveness to its homologous product and a feeble one for tuberculin. Attempts to sensitize normal guinea pigs intraperitoneally to nonspecific products failed to induce tuberculin skin reactions. The cutireaction of Pirquet is not suitable for application to guinea pigs, though preferable in man because of simplicity and innocuousness.—*Sur les propriétés antigènes et réactionelles des Tuberculines, F. De Potter, Rev. Belge d. l. Tuberculose, January–February, 1928, xix, 1.*—(*A. P.*)

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